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(57) Abstract

New peptides derived from the MAGE-2 molecule and which bind to HLA-A*0201 molecules are disclosed. Some of these are especially useful because, when complexed to their HLA-A*0201 partner molecules, they induce CTL proliferation.

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ISOLATED PEPTIDES DERIVED FROM MAGE-2

RELATED APPLICATIONS

This application is a continuation-in-part of Serial No. 08/217,188, filed March 24, 1994, now allowed.

FIELD OF THE INVENTION

This invention relates to immunogenetics and to peptide chemistry. More particularly, it relates to undecapeptides, decapeptides and nonapeptides useful in various ways, including immunogens and as ligands for the HLA-A2 molecule. More particularly, it relates to called "tumor rejection antigens", derived from the tumor rejection antigen precursor encoded by gene MAGE-2, and presented by the MHC-class I molecule HLA-A2.

BACKGROUND AND PRIOR ART

The study of the recognition or lack of recognition of cancer cells by a host organism has proceeded in many different directions. Understanding of the field presumes some understanding of both basic immunology and oncology.

Early research on mouse tumors revealed that these displayed molecules which led to rejection of tumor cells when transplanted into syngeneic animals. These molecules are "recognized" by T-cells in the recipient animal, and provoke a cytolytic T-cell response with lysis of the transplanted cells. This evidence was first obtained with tumors induced in vitro by chemical carcinogens, such as methylcholanthrene. The antigens expressed by the tumors and which elicited the T-cell response were found to be different for each tumor. See Prehn, et al., J. Natl. Canc. Inst. 18: 769-778 (1957); Klein et al., Cancer Res. 20: 1561-1572 (1960); Gross, Cancer Res. 3: 326-333 (1943), Basombrio, Cancer Res. 30: 2458-2462 (1970) for general teachings on inducing tumors with chemical carcinogens and differences in cell surface antigens. This class of antigens has come to be known as "tumor specific

transplantation antigens" or "TSTAs". Following the observation of the presentation of such antigens when induced by chemical carcinogens, similar results were obtained when tumors were induced in vitro via ultraviolet radiation. See Kripke, J. Natl. Canc. Inst. 53: 333-1336 (1974).

While T-cell mediated immune responses were observed for the types of tumor described supra, spontaneous tumors were thought to be generally non-immunogenic. These were therefore believed not to present antigens which provoked a response to the tumor in the tumor carrying subject. See Hewitt, et al., Brit. J. Cancer 33: 241-259 (1976).

The family of tum$^-$ antigen presenting cell lines are immunogenic variants obtained by mutagenesis of mouse tumor cells or cell lines, as described by Boon et al., J. Exp. Med. 152: 1184-1193 (1980), the disclosure of which is incorporated by reference. To elaborate, tum$^-$ antigens are obtained by mutating tumor cells which do not generate an immune response in syngeneic mice and will form tumors (i.e., "tum$^+$" cells). When these tum$^+$ cells are mutagenized, they are rejected by syngeneic mice, and fail to form tumors (thus "tum$^-$"). See Boon et al., Proc. Natl. Acad. Sci. USA 74: 272 (1977), the disclosure of which is incorporated by reference. Many tumor types have been shown to exhibit this phenomenon. See, e.g., Frost et al., Cancer Res. 43: 125 (1983).

It appears that tum$^-$ variants fail to form progressive tumors because they initiate an immune rejection process. The evidence in favor of this hypothesis includes the ability of "tum$^-$" variants of tumors, i.e., those which do not normally form tumors, to do so in mice with immune systems suppressed by sublethal irradiation, Van Pel et al., Proc. Natl. Acad. Sci. USA 76: 5282-5285 (1979); and the observation that intraperitoneally injected tum$^-$ cells of mastocytoma P815 multiply exponentially for 12-15 days, and then are eliminated in only a few days in the midst of an influx of lymphocytes and macrophages (Uyttenhove et al., J. Exp. Med. 152: 1175-1183 (1980)). Further evidence includes the observation that mice acquire an immune memory which permits them to resist

subsequent challenge to the same tum⁻ variant, even when immunosuppressive amounts of radiation are administered with the following challenge of cells (Boon et al., Proc. Natl, Acad. Sci. USA 74: 272-275 (1977); Van Pel et al., supra; Uyttenhove et al., supra). Later research found that when spontaneous tumors were subjected to mutagenesis, immunogenic variants were produced which did generate a response. Indeed, these variants were able to elicit an immune protective response against the original tumor. See Van Pel et al., J. Exp. Med. 157: 1992-2001 (1983). Thus, it has been shown that it is possible to elicit presentation of a so-called "tumor rejection antigen" in a tumor which is a target for a syngeneic rejection response. Similar results have been obtained when foreign genes have been transfected into spontaneous tumors. See Fearon et al., Cancer Res. 48: 2975-1980 (1988) in this regard.

A class of antigens has been recognized which are presented on the surface of tumor cells and are recognized by cytolytic T cells, leading to lysis. This class of antigens will be referred to as "tumor rejection antigens" or "TRAs" hereafter. TRAs may or may not elicit antibody responses. The extent to which these antigens have been studied, has been via cytolytic T cell characterization studies, in vitro i.e., the study of the identification of the antigen by a particular cytolytic T cell ("CTL" hereafter) subset. The subset proliferates upon recognition of the presented tumor rejection antigen, and the cells presenting the tumor rejection antigens are lysed. Characterization studies have identified CTL clones which specifically lyse cells expressing the tumor rejecton antigens. Examples of this work may be found in Levy et al., Adv. Cancer Res. 24: 1-59 (1977); Boon et al., J. Exp. Med. 152: 1184-1193 (1980); Brunner et al., J. Immunol. 124: 1627-1634 (1980); Maryanski et al., Eur. J. Immunol. 124: 1627-1634 (1980); Maryanski et al., Eur. J. Immunol. 12: 406-412 (1982); Palladino et al., Canc. Res. 47: 5074-5079 (1987). This type of analysis is required for other types of antigens recognized by CTLs, including minor histocompatibility

antigens, the male specific H-Y antigens, and the class of antigens referred to as "tum-" antigens, and discussed herein.

A tumor exemplary of the subject matter described supra is known as P815. See DePlaen et al., Proc. Natl. Acad. Sci. USA 85: 2274-2278 (1988); Szikora et al., EMBO J 9: 1041-1050 (1990), and Sibille et al., J. Exp. Med. 172: 35-45 (1990), the disclosures of which are incorporated by reference. The P815 tumor is a mastocytoma, induced in a DBA/2 mouse with methylcholanthrene and cultured as both an in vitro tumor and a cell line. The P815 line has generated many tum$^-$ variants following mutagenesis, including variants referred to as P91A (DePlaen, supra), 35B (Szikora, supra), and P198 (Sibille, supra). In contrast to tumor rejection antigens - and this is a key distinction - the tum$^-$ antigens are only present after the tumor cells are mutagenized. Tumor rejection antigens are present on cells of a given tumor without mutagenesis. Hence, with reference to the literature, a cell line can be tum$^+$, such as the line referred to as "P1", and can be provoked to produce tum$^-$ variants. Since the tum$^-$ phenotype differs from that of the parent cell line, one expects a difference in the DNA of tum$^-$ cell lines as compared to their tum$^+$ parental lines, and this difference can be exploited to locate the gene of interest in tum$^-$ cells. As a result, it was found that genes of tum$^-$ variants such as P91A, 35B and P198 differ from their normal alleles by point mutations in the coding regions of the gene. See Szikora and Sibille, supra, and Lurquin et al., Cell 58: 293-303 (1989). This has proved not to be the case with the TRAs of this invention. These papers also demonstrated that peptides derived from the tum$^-$ antigen are presented by the L^d molecule for recognition by CTLs. P91A is presented by L^d, P35 by D^d and P198 by K^d.

PCT application PCT/US92/04354, filed on May 22, 1992 assigned to the same assignee as the subject application, teaches a family of human tumor rejection antigen precursor coding genes, referred to as the MAGE family. Several of these genes are also discussed in van der Bruggen et al., Science 254: 1643 (1991). It is now clear that the various

genes of the MAGE family are expressed in tumor cells, and can serve as markers for the diagnosis of such tumors, as well as for other purposes discussed therein. See also Traversari et al., Immunogenetics 35: 145 (1992); van der Bruggen et al., Science 254: 1643 (1991) and De Plaen, et al., Immunogenetics 40: 360 (1994). The mechanism by which a protein is processed and presented on a cell surface has now been fairly well documented. A cursory review of the development of the field may be found in Barinaga, "Getting Some 'Backbone': How MHC Binds Peptides", Science 257: 880 (1992); also, see Fremont et al., Science 257: 919 (1992); Matsumura et al., Science 257: 927 (1992); Latron et al., Science 257: 964 (1992). These papers generally point to a requirement that the peptide which binds to an MHC/HLA molecule be nine amino acids long (a "nonapeptide"), and to the importance of the first and ninth residues of the nonapeptide.

Studies on the MAGE family of genes have now revealed that a particular nonapeptide is in fact presented on the surface of some tumor cells, and that the presentation of the nonapeptide requires that the presenting molecule be HLA-A1. Complexes of the MAGE-1 tumor rejection antigen (the "TRA" or nonapeptide") leads to lysis of the cell presenting it by cytolytic T cells ("CTLs").

Attention is drawn, e.g., to concurrently filed application Serial No. 08/217,187 to Traversari et al., and Serial No. 08/217,186 to Townsend et al., both of which present work on other, MAGE-derived peptides.

Research presented in, e.g., U.S. patent application Serial No. 07/938,334 filed August 31, 1992, and in U.S. patent application Serial No. 073,103, filed June 7, 1993, found that when comparing homologous regions of various MAGE genes to the region of the MAGE-1 gene coding for the relevant nonapeptide, there is a great deal of homology. Indeed, these observations lead to one of the aspects of the invention disclosed and claimed therein, which is a family of nonapeptides all of which have the same N-terminal and C-terminal amino acids. These nonapeptides were described as

being useful for various purposes which includes their use as immunogens, either alone or coupled to carrier peptides. Nonapeptides are of sufficient size to constitute an antigenic epitope, and the antibodies generated thereto were described as being useful for identifying the nonapeptide, either as it exists alone, or as part of a larger polypeptide.

These references, especially Serial No. 073,103, showed a connection between HLA-A1 and MAGE-3; however, only about 26% of the caucasian population and 17% of the negroid population presents HLA-A1 molecules on cell surfaces. Thus, it would be useful to have additional information on peptides presented by other types of MHC molecules, so that appropriate portions of the population may benefit from the research discussed supra.

It has now been found that antigen presentation of MAGE-2 derived peptides set forth, in the disclosure which follows, identifies peptides which complex with MHC class I molecule HLA-A2. The ramifications of this discovery, which include therapeutic and diagnostic uses, are among the subjects of the invention, set forth in the disclosure which follows.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is an exemplary graph showing the calculation of peptide concentration which includes 0.5 maximum upregulation of HLA-A2.1.

Figure 2 presents comparative data on the response of HPV clones to various materials, as measured by ^{51}Cr release assay.

Figures 3A-3D show results from positive bulk culture assays.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

EXAMPLE 1

Experimental conditions:

All experiments were performed at room temperature unless stated otherwise. All Fmoc protected amino acids, synthesis polymers, peptides and TFA were stored at -20°C.

Peptide synthesis

Peptides were synthesized by solid phase strategies on an automated multiple peptide synthesizer (Abimed AMS 422) (see Gausepohl and Frank, Biotech, Sept. 1990; Gausepohl et al. in E. Giralt and D. Andreu (eds), Peptides 1990: 206-207 (1990).

The peptides were made in various runs, in each of which 48 different peptides were synthesized simultaneously.

Tentagel S AC (Rapp et al., in Innovation and Perspective in Solid Phase Peptide Synthesis, 205-210 (1990); Sheppard and Williams, Int. J. Peptide Protein Res. 20: 451-454 (1982)), a graft polymer of polyethyleneglycol spacer arms on a polystyrene matrix, was used as a resin (40-60 mg per peptide, 10 μmol Fmoc amino acid loading).

Repetitive couplings were performed by adding a mixture of 90 μl 0.67 M BOP (Gausepohl et al., Peptides 241-243 (1988); Castro et al., Tett. Lett. 14: 1219-1222 (1975) in NMP, 20 μl NMM in NMP 2/1 (v/v) and 100 μl of an 0.60 M solution of the appropriate Fmoc amino acid (Fields and Noble, Int. J. Pep. Prot. Res. 35: 161-214 (1990)) in NMP (6-fold excess) to each reaction vessel. At 70% of the reaction time

approximately 50 µl dichloromethane was added to each reaction vessel.

Fmoc-deprotection was performed by adding 3 times 0.8 ml of piperidine/DMA 1/4 (v/v) to each reaction vessel.

Coupling- and deprotection times were increased as the synthesis proceeded, starting with 30 min and 3 times 3 min respectively.

Washings after couplings and Fmoc-deprotections were done with 6 times 1.2 ml DMA. After the required sequence had been reached and the last Fmoc-protection was removed the peptidylresin was washed extensively with DMA, dichloromethane, dichloromethane/ether 1/1 (v/v) and ether respectively, and dried.

Peptide cleavage and isolation

Cleavage of the petides from the resin and removal of the side chain protecting groups was performed by adding 6 times 200 µl TFA/water 19/1 (v/v) at 5 min intervals to each reaction vessel, thus yielding free carboxylic peptides. For Trp-containing peptides TFA/water/ethanethiol 18/1/1/ (v/v/v) was used.

Two hours after the first TFA addition to the peptides were precipitated from the combined filtrates by addition of 10 ml ether/pentane 1/1 (v/v) and cooling to -20°C. The peptides were isolated by centrifugation (-20°C, 2500g, 10 min).

After treatment of the pellet with ether/pentane 1/1 (v/v) and isolation by the same centrifugation procedure, the

peptides were dried at 45°C for 15 min.

Each of the peptides was dissolved in 2 ml water (or 2 ml 10 vol.% acetic acid), the solution frozen in liquid nitrogen for 3 min, and lyophilized while being centrifuged (1300 rpm, 8-16 h).

Analysis and purification

The purity of the peptides was determined by reversed phase HPLC; an aliquot of about 50 nmol was dissolved in 100 µl 30 vol.% acetic acid. Of this solution 30 µl was applied to an RP-HPLC system equipped with a ternary solvent system; A: water, B: acetonitrile, C: 2 vol.% TFA in water.

Gradient elution (1.0 ml/min) was performed from 90% A, 5% B, 5% C to 20% A, 75% B, 5% C in 30 min. Detection was at 214 nm.

Samples taken at random were analyzed by mass spectrometry on a PDMS. The 31 binding peptides were all analyzed by mass spectrometry on a PDMS and by quantitative amino acid analysis after hydrolysis on a HP Aminoquant. Of all analyzed samples the difference between calculated and measured masses was within the experimental error (0.1%) as specified by the producer of the equipment used. All amino acid compositions were as expected.

EXAMPLE 2

Peptides

Of all 71 MAGE-2 peptides that had been freeze dried, 1 mg was weighed and dissolved in 10 µl of DMSO. Of all dissolved peptides a dilution of 0.5 mg/ml in 0.9% NaCl was made and the pH was neutralized to pH 7 with 5% acetic acid diluted in distilled water (CH_3COOH, Merck Darmstadt, Germany) or 1N NaOH diluted in distilled water (Merck Darmstadt, Germany).

Cells

174CEM.T2 cells were cultured in Iscove's modified Dulbecco's medium (Biochrom KG Seromed Berlin, Germany) supplemented with 100IU/ml penicillin (Biocades Pharma, Leiderdorp, The Netherlands), 100 µg/ml kanamycin (Sigma St. Louis, USA), 2mM glutamine (ICN Biomedicals Inc. Costa Mesa, CA, USA) and 10% fetal calf serum (FCS, Hyclone Laboratories Inc. Logan, Utah, USA). Cells were cultured at a density of 2.5×10^5/ml during 3 days at 37°C, 5% CO_2 in humidified air.

Peptide binding

174CEM.T2 cells were washed twice in culture medium without FCS and put in serum-free culture medium to a density of 2×10^6 cells/ml. Of this suspension 40 µl was put into a V bottomed 96 well plate (Greiner GmbH, Frickenhausen, Germany) together with 10 µl of two fold serial dilutions in 0.9% NaCl of the individual peptide dilutions (ranging from 500 ug/ml to 15.6 µg/ml). The end concentrations range from

200 μg/ml to 3.1 μg/ml peptide with $8x10^4$ 174CEM.T2 cells. This solution was gently agitated for 3 minutes after which an incubation time of 16 hours at 37°C, 5% CO_2 in humified air took place. Then cells were washed once with 100 μl 0.9% NaCl, 0.5% bovine serum albumin (Sigma St. Louis, USA), 0.02% NaN_3 (Merck Darmstadt, Germany). After a centrifuge round of 1200 rpm the pellet was resuspended in 50 μl of saturating amounts of HLA-A2.1 specific mouse monoclonal antibody BB7.2 for 30 minutes at 4°C. Then cells were washed twice and incubated for 30 minutes with $F(ab)_2$ fragments of goat anti-mouse IgG that had been conjugated with fluorescein isothiocyanate (Tago Inc. Burlingame, CA, USA) in a dilution of 1:40 and a total volume of 25 μl.

After the last incubation, cells were washed twice and fluorescence was measured at 488 nanometer on a FACScan flowcytometer (Becton Dickinson, Franklin Lakes, NJ, USA). The concentration at which the 0.5 maximum upregulation of HLA-A2.1 on 174CEM.T2 cells was achieved was determined using graphs in which the fluorescence index was plotted against the peptide concentration. The results are shown in Table I.

TABLE I

Binding affinities of peptides derived from human melanoma associated protein MAGE-2 that fit the HLA-A2.1 motif (compilation of Falk et al., 1991, Hunt et al., 1992 and Nijman et al., 1993).

Peptide No.	sequence	residues	peptide concentration that induces 0.5 maximum FI
	GLEARGEALGL	15- 25	>100 µg/ml
	GLEARGEAL	15- 23	60 µg/ml4
	ALGLVGAQA	22- 30	>100 µg/ml
	GLVGAQAPA	24- 32	65 µg/ml
	DLESEFQAA	100-108	>100 µg/ml
	DLESEFQAAI	100-109	>100 µg/ml
	AISRKMVELV	108-117	>100 µg/ml
	AISRKMVEL	108-116	>100 µg/ml
2	KMVELVEFL	112-120	40 µg/ml
	KMVELVEFLL	112-121	>100 µg/ml
	KMVELVEFLLL	112-122	>100 µg/ml
	LLLKYRAREPV	120-130	>100 µg/ml
	LLKYRAREPV	121-130	>100 µg/ml
	VLRNCQDFFPV	139-149	>100 µg/ml
3	VIFSKASEYL	149-158	35 µg/ml
4	YLQLVFGIEV	157-166	35 µg/ml
	YLQLVFGIEVV	157-167	>100 µg/ml
5	QLVFGIEVV	159-167	25 µg/ml
6	QLVFGIEVVEV	159-169	30 µg/ml
	GIEVVEVVPI	163-172	>100 µg/ml
	PISHLYILV	171-179	55 µg/ml
	HLYILVTCL	174-182	>100 µg/ml
	HLYILVTCLGL	174-184	>100 µg/ml
	YILVTCLGL	176-184	>100 µg/ml
	CLGLSYDGL	181-189	65 µg/ml
	CLGLSYDGLL	181-190	>100 µg/ml
	VMPKTGLLI	195-203	>100 µg/ml
	VMPKTGLLII	195-204	>100 µg/ml
	VMPKTGLLIIV	195-205	>100 µg/ml
	GLLIIVLAI	200-208	>100 µg/ml
	GLLIIVLAII	200-209	>100 µg/ml
	GLLIIVLAIIA	200-210	>100 µg/ml
	LLIIVLAII	201-209	>100 µg/ml
	LLIIVLAIIA	201-210	>100 µg/ml
	LLIIVLAIIAI	201-211	>100 µg/ml
	LIIVLAIIA	202-210	>100 µg/ml
	LIIVLAIIAI	202-211	>100 µg/ml
7	IIVLAIIAI	203-211	20 µg/ml
	IIAIEGDCA	208-216	>100 µg/ml
	KIWEELSML	220-228	>100 µg/ml
8	KIWEELSMLEV	220-230	25 µg/ml
	LMQDLVQENYL	246-256	>100 µg/ml
	FLWGPRALI	271-279	65 µg/ml
9	ALIETSYVKV	277-286	20 µg/ml
	ALIETSYVKVL	277-287	>100 µg/ml
10	LIETSYVKV	278-286	30 µg/ml
	LIETSYVKVL	278-287	55 µg/ml
	TLKIGGEPHI	290-299	>100 µg/ml
	HISYPPLHERA	298-308	>100 µg/ml

The 174CEM.T2 cell line expresses "empty" and unstable HLA-A2.1 molecule that can be stabilized when a peptide is binding to the peptide presenting groove of these molecules. A stabilized HLA-A2.1 molecule that will not easily degrade is the result of binding of an analyzed peptide. This leads to an increase in cell surface expression of the HLA-A2.1 molecule. The fluorescence index is a measure for the amount of upregulation of HLA-A2.1 molecules. This fluorescence index is calculated according to the following formula:

MF = Mean Fluorescence

$$FI = \text{Fluorescence Index} = \frac{(MF)_{experiment} - (MF)_{blank}}{(MF)_{blank}}$$

Fluorescence Index of the background fluorescence is 0.

Results

In order to identify MAGE-2 peptides that could bind to HLA-A2.1 molecules expressed by 174CEM.T2 cells, the amino acid sequence of MAGE-2 was examined in accordance with van der Bruggen, et al., Science 254: 1643-1647 (1991). All peptides of nine, ten or eleven amino acids that fitted the published HLA-A2.1 binding motif were examined (Table I).

Only the peptides of SEQ ID NOS: 1-11 of Table III were able to upregulate the expression of HLA-A2.1 molecules at low peptide concentration, indicating their binding to the HLA-A2.1 molecule as described in Example 2. None of the 50 other peptides were able to do this. The results of the fluorescence measurement are given in Tables I and II. The 0.5 maximum upregulation of HLA-A2.1 molecules on 174CEM.T2 cells was determined using graphs in which the FI was plotted

against the peptide concentration for each individual peptide.

These experiments indicate that only a limited proportion of peptides that fit the HLA-A2.1 motif have the ability to bind to this HLA molecule with high affinity and are therefore likely candidates of MAGE-2 protein to be recognized by human CTL, which recognize peptides only when bound to HLA molecules.

TABLE II

Binding affinities of additional peptides derived from human melanoma associated protein MAGE-2 that fit the extended HLA-A2.1 motif (Ruppert et al, Cell 74: 929-937).

Peptide No.	Sequence	residues	peptide concentration that induces 0.5 maximum FI
	QTASSSSTL	37-45	>100 µg/ml
	QTASSSSTLV	37-46	>100 µg/ml
1	STLVEVTLGEV	43-53	45 µg/ml
	VTLGEVPAA	48-56	>100 µg/ml
	VTKAEMLESV	130-139	70 µg/ml
	VTKAEMLESVL	130-140	>100 µg/ml
	VTCLGLSYDGL	179-189	>100 µg/ml
	KTGLLIIVL	198-206	65 µg/ml
	KTGLLIIVLA	198-207	80 µg/ml
	KTGLLIIVLAI	198-208	>100 µg/ml
	HTLKIGGEPHI	289-299	100 µg/ml

TABLE III

Peptides derived from melanoma protein MAGE-2 binding to HLA-A2.1

Peptide No.	Amino Acid Sequence	Region	SEQ ID NO
1	STLVEVTLGEV	residues 43-53	1
-	LVEVTLGEV	residues 45-53	2
2	KMVELVHFL	residues 112-120	3
3	VIFSKASEYL	residues 149-158	4
4	YLQLVFGIEV	residues 157-166	5
5	QLVFGIEVV	residues 159-167	6
6	QLVFGIEVVEV	residues 159-169	7
7	IIVLAIIAI	residues 203-211	8
8	KIWEELSMLEV	residues 220-230	9
9	ALIETSYVKV	residues 277-286	10
10	LIETSYVKV	residues 278-286	11

Most HLA-A2.1 binding peptides were found using the HLA-A2.1 motif, in accordance with Falk et al., Nature 351: 290-296 (1991); Hunt et al., Science 255: 1261-1263 (1992); and Nijman et al., J. Immunother 14: 121-126 (1993). Only one additional HLA-A2.1 peptide was found using the extended HLA-A2.1 motif of Ruppert et al., Cell 74: 929-937 (1993).

EXAMPLE 3

This example shows the in vitro induction of primary immune response. As an illustration for the possibility of inducing primary responses in general, including MAGE-2 peptides, such responses against HPV peptides using the processing defective cell line 174CEM.T2 are shown.

The expression of HLA-A2.1 cells (T2) is increased by incubating T2 cells in medium containing relevant peptide. T2 cells will present the relevant peptide bound to HLA-A2.1 in

high amount and therefore are good antigen presenting cells (APC). In the response inducing method described recently (Kast et al., J. Immunother 14: 115-120 (1993), the T2 cell line is used as APC and post-Ficoll mononuclear cells are used as responder cells.

Method

1) Peptide loading of HLA-A2.1 on T2

T2 cells in a concentration of 2 x 10^6 cells per ml were incubated for 13 hours at 37°C in a T 25 flask (Becton Dickinson, Falcon, Plymouth England) in serum-free IMDM (= Iscoves Modified Dulbecco's Medium: Biochrom KG, Seromed Berlin, Germany) with glutamine (2mM, ICN Biochemicals Inc., Costa Meisa, USA), antibiotics (100 IU/ml penicillin (Brocades Pharma, Leiderdorp, The Netherlands, 100 µg/ml kanamycin (Sigma, St. Louis, USA)) and the selected peptide, MLDLQPETT (SEQ ID NO: 62) in a concentration of 80 µg/ml.

2) Mitomycin C treatment of T2 antigen producing cells from HPV (APC)

These incubated T2 antigen producing cells cells were spun down and subsequently treated in a density of 20 x 10^6 cells/ml with Mitomycin C (50 µg/ml) in serum-free RPMI (Gibco Paisley Scotland) medium for one hour at 37°C. Hereafter the T2 cells were washed three times in RPMI.

3) Preparing for primary immune response induction

All wells of a 96-well-U-bottom plate (Costar, Cambridge,

USA) were filled with 100,000 Mitomycin C-treated T2 cells in 50 µl serum-free, complete RPMI medium (glutamine (2 mM, ICN Biochemicals Inc., costa Meisa, USA), penicillin (100 IU/ml, Brocades Pharma, Leiderdorp, The Netherlands), kanamycin (100 µg/ml, Sigma, St. Louis, USA)) and the peptide MLDLQPETT (SEQ ID NO: 21) at a concentration of 80 µg/ml.

4) Responder cells

Responder cells are mononuclear peripheral blood lymphocytes (PBL) of a HLA-A2.1 subtyped donor. The PBL were separated from buffy coats by Ficoll-procedure (Ficoll preparation: Lymphoprep of Nycomedpharma, Oslo, Norway) and washed two times in RPMI. After separation and washing, the PBL were resuspended in complete RPMI medium with 30% human pooled serum (HPS) (HPS was tested for a suppression activity in mixed lymphocyte cultures).

5) Incubation for primary immune response

400,000 PBLs in 50 µl of medium (the medium described in paragraph 4, supra) were added to each well of the 96-well-U-bottom plate already filled with T2 cells and cultured for 7 days at 37°C in an incubator with 5% CO_2 and 90% humidity.

6) Restimulation (day 7)

On day 7 after incubation of PBLs, peptide MLDLQPETT (SEQ ID NO: 21) and T2 cells described supra, the PBLs were restimulated with peptide MLDLQPETT (SEQ ID no: 62). For this purpose all cells and medium out of the 96 wells were

harvested. Viable cells were isolated by the Ficoll-procedure and washed in RPMI. In a new 96-well-U-bottom plate 50,000 of these viable cells were seeded in each well together with 50 µl complete RPMI medium with 15% HPS. Per well 20,000 autologous, irradiated (3000 rad) PBLs and 50,000 autologous, irradiated (10000 rad) EBV-transformed B-lymphocytes were added together with 50 µl of complete RPMI medium with 15% HPS and peptide MLDLQPETT (SEQ ID NO: 62) at a concentration of 80 µg/ml. The cells were cultured for 7 days at 37°C in an incubator with 5% CO_2 and 90% humidity.

7) Restimulation (day 14)

On day 14 after incubation of PBL, peptide MLDLQPETT (SEQ ID NO: 62) and T2 cells, PBLs were restimulated with peptide MLDLQPETT (SEQ ID NO: 62). To do so the procedure under point 6, supra is repeated.

8) Cloning by Limiting Dilution

On day 21 after incubation of PBL, peptide MLDLQPETT (SEQ ID NO: 62) and T2 cells, cells and medium out of the 96 wells were harvested. Viable cells were isolated by Ficoll-procedure and washed in complete RPMI with 15% HPS. This bulk culture of viable cells was cloned by limiting dilution. Into each well of a new 96-well-U-bottom plate (Costar, Cambridge, USA) 50 µl complete RPMI medium with 15% HPS was added together with 100 viable cells (= HPV16 bulk anti MLDLQPETT (SEQ ID NO: 62)). For other new 96-well-U-bottom plates this was exactly repeated except for the number of cells for wells:

subsequent plates contained dilutions of cells at 10, 1, or 0.3 cells per well. To all wells 20,000 pooled and irradiated (3000 rad) PBL of four different donors and 10,000 pooled and irradiated (10,000 rad) EBV-transformed B-cells of three different HLA-A2.1 donors (VU-4/518/JY) were added together with 50 µl of complete RPMI medium with 15% HPS and peptide MLDLQPETT (SEQ ID NO: 62) in a concentration of 40 µg/ml, Leucoagglutinin in a concentration of 2 ug/ml (Pharmacia, Uppsala, Sweden), human recombinant IL-2 in a concentration of 120 IU/ml (Eurocetus, Amsterdam, The Netherlands).

9) Expand clones

Add per well, in a final volume of 100 µl =>

- 25,000 viable cells
- 20,000 irradiated PBL-pool (described supra)
- 10,000 irradiated EBV-pool (described supra)
- 2 µg peptide MLDLQPETT (SEQ ID NO: 62)
- 6 IU recombinant IL-2.

On day 49 a cytotoxicity assay was performed with 65 clones and one bulk culture sample as effector cells and T2 (with or without the relevant peptide MLDLQPETT (SEQ ID NO: 62)) as target cells. Background killing is defined as killing of T2 cells incubated with an irrelevant (but HLA-A2.1 binding) peptide: GILGFVFTL (SEQ ID NO: 64). This influenza matrix protein-derived peptide is the epitope for HLA-A2.1 restricted influenza specific CTL and is known in the art.

The HPV bulk anti-SEQ ID NO: 62 effector cells seemed to

be specific for killing SEQ ID NO: 62 sensitive cells.

A limiting dilution assay was done with the HPV bulk culture cells and, after 23 days, a cytotoxicity assay was performed with five clones. Results of a representative clone are shown in Fig. 2

EXAMPLE 4

Several groups have reported on the requirements for binding of peptides to HLA-A*0201 molecules. Highly important (anchor), and important (dominant) residues have been elucidated by, e.g., Falk et al., Nature 351: 290 (1991); Nijman et al., Eur. J. Immunol. 23: 1215 (1993); Ruppert et al., Cell 74: 929 (1993), all of which are incorporated by reference. Using the data in these papers, and a screening program described by Drijfhout et al., Human Immunol. 43: 1 (1995); D'Amaro et al., Human Immunol. 43: 13 (1995), both of which are incorporated by reference, the deduced amino acid sequence for MAGE-2 was screened for putative binding peptides. A peptide was deemed to fit the reference motifs if one anchor and one dominant residue or two anchor residues were present. All of these peptides were synthesized, using well known solid phase synthesis techniques, and were then tested in a peptide binding competitive assay, in accordance with van der Burg et al., Human Immunol. 44: 189-198 (1995), incorporated by reference. In brief, the cell line JY, which is an EBV transformed B cell line homozygous for HLA-A*0201 was stripped of binding peptides via exposure to ice cold citric acid buffer (pH 3.2), for 90 seconds. (Buffer was

be specific for killing SEQ ID NO: 62 sensitive cells.

A limiting dilution assay was done with the HPV bulk culture cells and, after 23 days, a cytotoxicity assay was performed with five clones. Results of a representative clone are shown in Fig. 2

EXAMPLE 4

Several groups have reported on the requirements for binding of peptides to HLA-A*0201 molecules. Highly important (anchor), and important (dominant) residues have been elucidated by, e.g., Falk et al., Nature 351: 290 (1991); Nijman et al., Eur. J. Immunol. 23: 1215 (1993); Ruppert et al., Cell 74: 929 (1993), all of which are incorporated by reference. Using the data in these papers, and a screening program described by Drijfhout et al., Human Immunol. 43: 1 (1995); D'Amaro et al., Human Immunol. 43: 13 (1995), both of which are incorporated by reference, the deduced amino acid sequence for MAGE-2 was screened for putative binding peptides. A peptide was deemed to fit the reference motifs if one anchor and one dominant residue or two anchor residues were present. All of these peptides were synthesized, using well known solid phase synthesis techniques, and were then tested in a peptide binding competitive assay, in accordance with van der Burg et al., Human Immunol. 44: 189-198 (1995), incorporated by reference. In brief, the cell line JY, which is an EBV transformed B cell line homozygous for HLA-A*0201 was stripped of binding peptides via exposure to ice cold citric acid buffer (pH 3.2), for 90 seconds. (Buffer was

equal volumes of 0.263M citric acid, and 0.123M Na_2HPO_4). The stripped cells were then washed with IMDM, and were then resuspended by IMDM, supplemented with 1.5 ug/ml ß-microglobulin. A reference peptide, i.e.:

Phe Leu Pro Ser Asp Cys Phe Pro Ser Val

(SEQ ID NO: 63)

was used, labelled with fluorescein at the cysteine residue. In the assay, 150 nM of SEQ ID NO: 63 were placed in separate wells of a 96 well, U bottom plate, and had titrated amounts of test peptide added thereto. Samples of stripped JY cells ($7x10^5$ cells), were incubated with the peptides, for 24 hours, at 4°C. Cells were then washed with PBS containing 1% bovine serum albumin, and then fixed with PBS containing 10% paraformaldehyde, and analyzed for inhibition of binding of the fluorescent-labeled reference peptide.

An inhibition was determined by using the formula:

$$1 = \frac{(MF_{exp\ well} - MF_{background})}{(MF_{ref.peptide} - MF_{background})} \times 100$$

"MF background" refers to mean fluorescence values obtained without reference peptide. "MF ref. peptide" refers to mean fluorescence values obtained after incubation with only 150 nm of reference peptide. By plotting results of several serial dilutions of peptides in semi-logarithmic form, 50% inhibition ("IC_{50}") could be calculated. Table IV, which follows, presents some of these data. A SEQ ID NO: is provided when the peptide which was tested is one referred to in the prior examples. An asterisk (*) indicates that the IC_{50} was greater than 100 μM SEQ ID NOS: 71, 72 and 73 are all prior art

peptides which are known to bind the HLA-A*0201 molecule.

Table IV

PEPTIDE	IC_{50}
SEQ ID NO: 1	*
SEQ ID NO: 3	7
SEQ ID NO: 4	*
SEQ ID NO: 5	7
SEQ ID NO: 6	47
SEQ ID NO: 7	26
SEQ ID NO: 8	*
SEQ ID NO: 9	10
SEQ ID NO: 10	*
SEQ ID NO: 11	*
SEQ ID NO: 13	*
SEQ ID NO: 15	30
SEQ ID NO: 27	80
SEQ ID NO: 31	42
SEQ ID NO: 47	6
SEQ ID NO: 48	*
Thr Leu Val Glu Val Thr Leu Gly Glu Val (SEQ ID NO:64)	17
Leu Val Glu Val Thr Leu Gly Glu Val (SEQ ID NO:65)	*
Lys Ala Ser Glu Tyr Leu Gln Leu Val (SEQ ID NO:66)	14
Gln Val Met Pro Lys Thr Gly Leu Leu Ile Ile (SEQ ID NO:67)	82
Lys Thr Gly Leu Leu Ile Ile Val Leu (SEQ ID NO:68)	27
Phe Leu Trp Gly Pro Arg Ala Leu Ile Glu Thr (SEQ ID NO:69)	9
Phe Leu Pro Ser Asp Asp Phe Pro Ser Val (SEQ ID NO:70)	1
Gly Ile Leu Gly Phe Val Phe Thr Leu (SEQ ID NO:71)	3
Tyr Met Asn Gly Thr Met Ser Gln Val (SEQ ID NO:72)	9

It will be noted that besides the control only seven peptides were capable of inhibiting binding of the reference peptide, when used at low concentrations, i.e., SEQ ID NOS: 3, 5, 9, 47, 64, 66 and 69. These peptides were then tested in further experiments.

EXAMPLE 5

The experiments of example 4 were carried out at 4°C, which eliminates temperature as a factor implicated in complex stability. A second set of experiments were carried out, at the human phsiological temperature of 37°C. The methodology according to van der Burg, et al., J. Immunol. 156(1) 33087 is essentially as follows. JY cells, as described in example 4, were treated with emetine to stop protein synthesis. This prevents the cells from presenting newly synthesized HLA-A*0201 molecules on their surfaces. Then, the cells were stripped of any presented peptides via the use of mild acid treatment. They were then contacted with test peptides, at a concentration of 200 ug/ml. Peptide loaded cells were then washed with cold Iscover's modified Dulbecco's medium (IMDM), and incubated in IMDM at 37°C, starting at time=0, for 2, 4, and 6 hours. The amount of HLA-A*0201 peptide complexes present was measured by staining the cells with HLA-A2 conformation-specific monoclonal antibody BB 7.2, available from the American Type Culture Collection, and GaM-Fitc. The contacting steps were followed by FACScan analysis. Fluorescence Index was then calculated, using:

$$Fl = \frac{(MF_{sample} - MF_{background})}{MF_{background}}$$

where $MF_{background}$ is the value obtained without the peptides. Each sample was tested twice, and mean FI was calculated at each listed time point. Percentage of residual HLA-A2 molecules was calculated by finding FI at t=0, and then applying:

$$\%_{remaining\ (t=n)} = (FI_{t=n}/FI_{t=0}) \times 100$$

It is known that dissociation of peptides from MHC is a linear process. It is also known that the capacity of a peptide to form stable complexes for long periods of time is related to the immunogenicity of that papetide in vivo. (See, e.g., van der Burg, et al. supra). As such, the stability of peptides was measured at the time required for 50% of the molecules to decay, starting at t=2. This value is referred to as the "DT_{50}" value hereafter. Linear regression analysis of the sequential measurements plotted against the percentage of remaining HLA-A2 molecules permitted the DT_{50} to be calculated. Of the seven peptides listed supra, SEQ ID NOS: 3, 5 and 9 induced peptide-HLA-A*0201 complexes with a DT_{50} of over six hours at 37°C. The other peptides showed lower levels of affinity.

TABLE V

PEPTIDE	IC_{50}	DT_{50}
SEQ ID NO: 3	7	>6
SEQ ID NO: 5	7	>6
SEQ ID NO: 9	10	>6
SEQ ID NO: 47	6	3
SEQ ID NO: 64	17	4
SEQ ID NO: 66	14	3.5
SEQ ID NO: 69	9	5

Test peptides SEQ ID NOS: 71 and 72 both had DT_{50} values greater than 6.

EXAMPLE 6

The immunogenicity of the peptides listed supra was tested. In these experiments, transgenic, HLA-A*0201K^b mice were used. These mice express the product of the chimeric, HLA-A*0201K^b gene, wherein the α3 domain of HLA-A*0201 is replaced by the murine H-

$2K^b\alpha 3$ domain. The resulting molecules bind HLA-A*0201 molecules, and interact with murine $CD8^+$ cells.

The mice were used in groups of 2-3 animals. Each was injected in the flank with 50 μg of peptides, mixed with 140 μg of HBV core antigen-derived, T helper epitope, as described by Millich et al., Proc. Natl. Acad. Sci. USA 85: 1610 (1988), incorporated by reference, emulsified in Incomplete Freund's Adjuvant. The animals were boosted, 14 days later, with the same mixture. The mice were sacrificed 11-14 days after the last injection, their spleen cells were passed through nylon wool, and $3x10^7$ cell samples were restimulated, in vitro, with $1x10^7$ thoroughly washed, syngeneic peptide-loaded LPS-elicited lymphoblasts IMDM, supplemented with penicillin, 8% heat inactivated FCS, and 20 μM 2-mercaptoethanol in standing T25 tissue culture flasks. The cultures were incubated for six days at 37°C, in a 5% CO_2 humidified air atmosphere, and then cytolytic activity of these bulk cultures were tested. This involved a standard, ^{51}Cr or a fluorescent Europium release assay, in accordance with, e.g., De Waal et al., J. Immunol. 125: 2665 (1983); Bouma et al., Human Immunol. 35: 85 (1992). In brief, labelled target cells were loaded with 10 μg/ml of peptide for at least 20 minutes, at 37°C. Titrated amounts of effector cells were then incubated with equal amounts of target cells for at least four hours. Spontaneous and maximal release were measured in groups of six. A response is deemed positive when the lysis in a cytotoxicity assay of target cells, loaded with the specific peptide, is at least 10% higher at two E/T ratios, than the background lysis of unloaded target cells. Figures 3A-3D presents

the results of positive bulk cultures for SEQ ID NOS: 3, 5 and 9, as well as test peptide SEQ ID NO: 71. The remaining peptides were not immunogenic.

EXAMPLE 7

The bulk CTL cultures, referred to supra, were then tested in a TNF release assay. Specifically, COS-7 cells were transfected with HLA-A*0201K^b, MAGE-2, and/or tyrosinase cDNA cloned into pcDNAI/Amp, using the well known DEAE-dextran chloroquine method of Seed et al., Proc. Natl. Acad. Sci. USA 84: 3365 (1987). After 48 hours, medium was discarded, and the COS-7 cells were used as stimulator cells in a TNF release assay. In brief, 5x10^3 murine, bulk culture cells, or 2x10^3 human CTLs were added to transfected COS-7 cells. After 24 hours, supernatant was harvested and TNF content determined, using TNF sensitive WEHI 164 clone 13 cells.

Bulk cultures derived from mice immunized with the peptides SEQ ID NOS: 3 and 5 showed recognition of the COS-7 cells transfected with HLA-A*0201K^b and MAGE-2, indicating that these two peptides are processed and presented by HLA-A*0201.

The data suggest that the peptides of SEQ ID NOS: 1-11 are single polypeptides of identified sequences. However, homologs, isoforms or genetic variants of these peptides may exist within or outside the cellular environment. This invention encompasses all such homologs, isoforms or genetic variants of the above peptides provided that they bind to an HLA-A2 molecule.

Polypeptides that are homologs of the peptides specifically include those having amino acid sequences which are at least about

40% conserved in relation to the amino acid sequence set forth in Table II, preferentially at least about 60% conserved, and more preferentially at least about 75% conserved.

It will be understood by one of ordinary skill in the art that other variants of the peptides shown above are included with the scope of the present invention. This particularly includes any variants that differ from the above mentioned and synthesized peptides only by conservative amino acid substitution. In particular, replacements of C (cysteine) by A (alanine), S (serine), α-aminobutyric acid and others are included as it is known that cysteine-containing peptides are susceptible to (air) oxidation during synthesis and handling. Many such conservative amino acid substitutions are set forth as sets by Taylor, J. Mol. Biol. 188: 233-258 (1986).

Herein the peptides shown above or fragments thereof include any variation in the amino acid sequence, whether by conservative amino acid substitution, deletion, or other processes, provided that the polypeptides bind to an HLA-A2 molecule. The fragments of the peptides may be small peptides with sequences of as little as five or more amino acids, said sequence being those disclosed in Table II when said polypeptides bind to the HLA-A2.1 molecule.

Polypeptides larger than the peptides shown are especially included within the scope of the present invention when said polypeptides induce a MAGE-2 specific CTL response in HLA-A2.1 positive individuals and include a (partial) amino acid sequence as set forth in Table II, or conservative substitutions thereof. Such polypeptides may have a length of from 9 to 12, more preferably 9 to 11 or even 9 to 10 amino acids.

This invention includes the use of polypeptides generated by every means, whether genetic engineering, peptide synthesis with solid phase techniques or others. The foregoing peptides may have various chemical modifications made at the terminal ends and still be within the scope of the present invention. Also other chemical modifications are possible, particularly cyclic and dimeric configurations. The term "derivatives" intends to cover all such modified peptides.

The polypeptides of the present invention find utility for the prophylaxis, diagnosis, and/or treatment or prevention of diseases involving MAGE-2 expressing cells including melanomas cells and other cancer cells.

For all applications the peptides are administered in an immunogenic form. Since the peptides are relatively short, this may necessitate admixture, complexing, conjugation, or chemical with an immunogenicity conferring binding carrier material such as lipids or others or the use of adjuvants.

The magnitude of a prophylactic or a therapeutic dose of polypeptides of this invention will, of course, vary with the group of patients (age, sex, weight, etcetera), the nature of the severity of the condition to be treated, the particular polypeptide of this invention and its route of administration. Any suitable route of administration may be employed to achieve an effective dosage of a polypeptide identified by this invention, as well as any dosage form well known in the art of pharmacy. In addition the polypeptides may also be administered by controlled release means and/or delivery devices. They may also be administered in combination with other active substances, such as,

in particular, T-cell activating agents like interleukin-2 etc.

The peptides of this invention may also be useful for other purposes, such as diagnostic use. For example, they may be used to check whether a vaccination with a peptide according to the invention has been successful. This may be done in vitro by testing whether said peptide is able to activate T cells of the vaccinated person.

As noted, supra, isolated cytolytic T cell clones ("CTLs") specific for complexes of HLA-A2 molecules, such as HLA-A*0201 and particular peptides, and method for making these in vivo are also contemplated. "Making" in this context essentially means stimulation proliferation of the CTLs by the presentation of a particular peptide by the HLA-A2 molecule. This can be done, e.g., by using subjects in need of additional CTLs.

Not all complexes of peptide and HLA-A2 molecule will lead to CTL proliferation; however, the specificity of the peptides for their target HLA-A*0201 molecules makes them useful, nonetheless, as diagnostic markers to type a cell as HLA-A2 positive or not.

Other aspects of the invention will be clear to the skilled artisan, and need not be repeated here.

The terms and expressions which have been employed are used as terms of description and not of limitation, and there is no intention in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, it being recognized that various modifications are possible within the scope of the invention.

(1) GENERAL INFORMATION:

(i) APPLICANTS:

(ii) TITLE OF INVENTION: Isolated Peptides Derived From MAGE-2, Cytolytic T Cells Specific To Complexes of Peptide and HLA-A2 Molecules, and Uses Thereof

(iii) NUMBER OF SEQUENCES: 69

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(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Diskette, 5.25 inch, 360 kb storage
(B) COMPUTER: IBM PS/2
(C) OPERATING SYSTEM: PC-DOS
(D) SOFTWARE: Wordperfect

(vi) CURRENT APPLICATION DATA:
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(2) INFORMATION FOR SEQUENCE ID NO: 1:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acid residues
(B) TYPE: amino acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

Ser Thr Leu Val Glu Val Thr Leu Gly Glu Val
1 5 10

(2) INFORMATION FOR SEQUENCE ID NO: 2:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acid residues
(B) TYPE: amino acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Leu Val Glu Val Thr Leu Gly Glu Val
1 5

(2) INFORMATION FOR SEQUENCE ID NO: 3:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acid residues
(B) TYPE: amino acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

Lys Met Val Glu Leu Val His Phe Leu

1 5

(2) INFORMATION FOR SEQUENCE ID NO: 4:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acid residues
(B) TYPE: amino acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

Val Ile Phe Ser Lys Ala Ser Glu Tyr Leu
1 5 10

(2) INFORMATION FOR SEQUENCE ID NO: 5:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acid residues
(B) TYPE: amino acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

Tyr Leu Gln Leu Val Phe Gly Ile Glu Val
1 5 10

(2) INFORMATION FOR SEQUENCE ID NO: 6:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acid residues
(B) TYPE: amino acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

Gln Leu Val Phe Gly Ile Glu Val Val

1 5

(2) INFORMATION FOR SEQUENCE ID NO: 7:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acid residues
(B) TYPE: amino acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

Gln Leu Val Phe Gly Ile Glu Val Val Glu Val

1 5 10

(2) INFORMATION FOR SEQUENCE ID NO: 8:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acid residues
(B) TYPE: amino acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

Ile Ile Val Leu Ala Ile Ile Ala Ile

1 5

(2) INFORMATION FOR SEQUENCE ID NO: 9:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acid residues
(B) TYPE: amino acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

Lys Ile Trp Glu Glu Leu Ser Met Leu Glu Val

1 5 10

(2) INFORMATION FOR SEQUENCE ID NO: 10:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acid residues
(B) TYPE: amino acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

Ala Leu Ile Glu Thr Ser Tyr Val Lys Val

1 5 10

(2) INFORMATION FOR SEQUENCE ID NO: 11:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acid residues
(B) TYPE: amino acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

Leu Ile Glu Thr Ser Tyr Val Lys Val
1 5

(2) INFORMATION FOR SEQUENCE ID NO: 12:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acid residues
(B) TYPE: amino acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

Gly Leu Glu Ala Arg Gly Glu Ala Leu Gly Leu

1 5 10

(2) INFORMATION FOR SEQUENCE ID NO: 13:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acid residues
(B) TYPE: amino acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

Gly Leu Glu Ala Arg Gly Glu Ala Leu

1 5

(2) INFORMATION FOR SEQUENCE ID NO: 14:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acid residues
(B) TYPE: amino acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

Ala Leu Gly Leu Val Gly Ala Gln Ala

1 5

(2) INFORMATION FOR SEQUENCE ID NO: 15:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acid residues
(B) TYPE: amino acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

Gly Leu Val Gly Ala Gln Ala Pro Ala

1 5

(2) INFORMATION FOR SEQUENCE ID NO: 16:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acid residues
(B) TYPE: amino acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

Asp Leu Glu Ser Glu Phe Gln Ala Ala

1 5

(2) INFORMATION FOR SEQUENCE ID NO: 17:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acid residues
(B) TYPE: amino acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

Asp Leu Glu Ser Glu Phe Gln Ala Ala Ile
1 5 10

(2) INFORMATION FOR SEQUENCE ID NO: 18:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acid residues
(B) TYPE: amino acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

Ala Ile Ser Arg Lys Met Val Glu Leu Val
1 5 10

(2) INFORMATION FOR SEQUENCE ID NO: 19:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acid residues
(B) TYPE: amino acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

Ala Ile Ser Arg Lys Met Val Glu Leu
1 5

(2) INFORMATION FOR SEQUENCE ID NO: 20:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acid residues
(B) TYPE: amino acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

Lys Met Val Glu Leu Val His Phe Leu Leu
1 5 10

(2) INFORMATION FOR SEQUENCE ID NO: 21:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acid residues
(B) TYPE: amino acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

Lys Met Val Glu Leu Val His Phe Leu Leu Leu
1 5 10

(2) INFORMATION FOR SEQUENCE ID NO: 22:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acid residues
(B) TYPE: amino acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

Leu Leu Leu Lys Tyr Arg Ala Arg Glu Pro Val
1 5 10

(2) INFORMATION FOR SEQUENCE ID NO: 23:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acid residues
(B) TYPE: amino acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

Leu Leu Lys Tyr Arg Ala Arg Glu Pro Val
1 5 10

(2) INFORMATION FOR SEQUENCE ID NO: 24:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acid residues
(B) TYPE: amino acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

Val Leu Arg Asn Cys Gln Asp Phe Phe Pro Val
1 5 10

(2) INFORMATION FOR SEQUENCE ID NO: 25:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acid residues
(B) TYPE: amino acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

Tyr Leu Gln Leu Val Phe Gly Ile Glu Val Val
1 5 10

(2) INFORMATION FOR SEQUENCE ID NO: 26:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acid residues
(B) TYPE: amino acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

Gly Ile Glu Val Val Glu Val Val Pro Ile
1 5 10

(2) INFORMATION FOR SEQUENCE ID NO: 27:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acid residues
(B) TYPE: amino acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

Pro Ile Ser His Leu Tyr Ile Leu Val
1 5

(2) INFORMATION FOR SEQUENCE ID NO: 28:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acid residues
(B) TYPE: amino acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

His Leu Tyr Ile Leu Val Thr Cys Leu
1 5

(2) INFORMATION FOR SEQUENCE ID NO: 29:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acid residues
(B) TYPE: amino acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 29:

His Leu Tyr Ile Leu Val Thr Cys Leu Gly Leu
1 5 10

(2) INFORMATION FOR SEQUENCE ID NO: 30:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acid residues
(B) TYPE: amino acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 30:

Tyr Ile Leu Val Thr Cys Leu Gly Leu
1 5

(2) INFORMATION FOR SEQUENCE ID NO: 31:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acid residues
(B) TYPE: amino acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 31:

Cys Leu Gly Leu Ser Tyr Asp Gly Leu
1 5

(2) INFORMATION FOR SEQUENCE ID NO: 32:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acid residues
(B) TYPE: amino acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 32:

Cys Leu Gly Leu Ser Tyr Asp Gly Leu Leu
1 5 10

(2) INFORMATION FOR SEQUENCE ID NO: 33:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acid residues
(B) TYPE: amino acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 33:

Val Met Pro Lys Thr Gly Leu Leu Ile
1 5

(2) INFORMATION FOR SEQUENCE ID NO: 34:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acid residues
(B) TYPE: amino acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 34:

Val Met Pro Lys Thr Gly Leu Leu Ile Ile
1 5 10

(2) INFORMATION FOR SEQUENCE ID NO: 35:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acid residues
(B) TYPE: amino acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 35:

Val Met Pro Lys Thr Gly Leu Leu Ile Ile Val
1 5 10

(2) INFORMATION FOR SEQUENCE ID NO: 36:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acid residues
(B) TYPE: amino acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 36:

Gly Leu Leu Ile Ile Val Leu Ala Ile
1 5

(2) INFORMATION FOR SEQUENCE ID NO: 37:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acid residues
(B) TYPE: amino acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 37:

Gly Leu Leu Ile Ile Val Leu Ala Ile Ile
1 5 10

(2) INFORMATION FOR SEQUENCE ID NO: 38:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acid residues
(B) TYPE: amino acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 38:

Gly Leu Leu Ile Ile Val Leu Ala Ile Ile Ala
1 5 10

(2) INFORMATION FOR SEQUENCE ID NO: 39:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acid residues
(B) TYPE: amino acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 39:

Leu Leu Ile Ile Val Leu Ala Ile Ile
1 5

(2) INFORMATION FOR SEQUENCE ID NO: 40:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acid residues
(B) TYPE: amino acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 40:

Leu Leu Ile Ile Val Leu Ala Ile Ile Ala
1 5 10

(2) INFORMATION FOR SEQUENCE ID NO: 41:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acid residues
(B) TYPE: amino acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 41:

Leu Leu Ile Ile Val Leu Ala Ile Ile Ala Ile
1 5 10

(2) INFORMATION FOR SEQUENCE ID NO: 42:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acid residues
(B) TYPE: amino acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 42:

Leu Ile Ile Val Leu Ala Ile Ile Ala
1 5

(2) INFORMATION FOR SEQUENCE ID NO: 43:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acid residues
(B) TYPE: amino acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 43:

Leu Ile Ile Val Leu Ala Ile Ile Ala Ile
1 5 10

(2) INFORMATION FOR SEQUENCE ID NO: 44:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acid residues
(B) TYPE: amino acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 44:

Ile Ile Ala Ile Glu Gly Asp Cys Ala
1 5

(2) INFORMATION FOR SEQUENCE ID NO: 45:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acid residues
(B) TYPE: amino acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 45:

Lys Ile Trp Glu Glu Leu Ser Met Leu
1 5

(2) INFORMATION FOR SEQUENCE ID NO: 46:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acid residues
(B) TYPE: amino acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 46:

Leu Met Gln Asp Leu Val Gln Glu Asn Tyr Leu
1 5 10

(2) INFORMATION FOR SEQUENCE ID NO: 47:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acid residues
(B) TYPE: amino acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 47:

Phe Leu Trp Gly Pro Arg Ala Leu Ile
1 5

(2) INFORMATION FOR SEQUENCE ID NO: 48:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acid residues
(B) TYPE: amino acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 48:

Leu Ile Glu Thr Ser Tyr Val Lys Val Leu
1 5 10

(2) INFORMATION FOR SEQUENCE ID NO: 49:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acid residues
(B) TYPE: amino acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 49:

Ala Leu Ile Glu Thr Ser Tyr Val Lys Val Leu
1 5 10

(2) INFORMATION FOR SEQUENCE ID NO: 50:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acid residues
(B) TYPE: amino acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 50:

Thr Leu Lys Ile Gly Gly Glu Pro His Ile
1 5 10

(2) INFORMATION FOR SEQUENCE ID NO: 51:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acid residues
(B) TYPE: amino acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 51:

His Ile Ser Tyr Pro Pro Leu His Glu Arg Ala
1 5 10

(2) INFORMATION FOR SEQUENCE ID NO: 52:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acid residues
(B) TYPE: amino acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 52:

Gln Thr Ala Ser Ser Ser Ser Thr Leu
1 5

(2) INFORMATION FOR SEQUENCE ID NO: 53:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acid residues
(B) TYPE: amino acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 53:

Gln Thr Ala Ser Ser Ser Ser Thr Leu Val
1 5 10

(2) INFORMATION FOR SEQUENCE ID NO: 54:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acid residues
(B) TYPE: amino acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 54:

Val Thr Leu Gly Glu Val Pro Ala Ala
1 5

(2) INFORMATION FOR SEQUENCE ID NO: 55:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acid residues
(B) TYPE: amino acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 55:

Val Thr Lys Ala Glu Met Leu Glu Ser Val
1 5 10

(2) INFORMATION FOR SEQUENCE ID NO: 56:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acid residues
(B) TYPE: amino acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 56:

Val Thr Lys Ala Glu Met Leu Glu Ser Val Leu
1 5 10

(2) INFORMATION FOR SEQUENCE ID NO: 57:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acid residues
(B) TYPE: amino acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 57:

Val Thr Cys Leu Gly Leu Ser Tyr Asp Gly Leu
1 5 10

(2) INFORMATION FOR SEQUENCE ID NO: 58:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acid residues
(B) TYPE: amino acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 58:

Lys Thr Gly Leu Leu Ile Ile Val Leu
1 5

(2) INFORMATION FOR SEQUENCE ID NO: 59:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acid residues
(B) TYPE: amino acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 59:

Lys Thr Gly Leu Leu Ile Ile Val Leu Ala
1 5 10

(2) INFORMATION FOR SEQUENCE ID NO: 60:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acid residues
(B) TYPE: amino acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 60:

Lys Thr Gly Leu Leu Ile Ile Val Leu Ala Ile
1 5 10

(2) INFORMATION FOR SEQUENCE ID NO: 61:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acid residues
(B) TYPE: amino acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 61:

His Thr Leu Lys Ile Gly Gly Glu Pro His Ile
1 5 10

(2) INFORMATION FOR SEQUENCE ID NO: 62:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acid residues
(B) TYPE: amino acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 62:

Met Leu Asp Leu Gln Pro Glu Thr Thr
1 5

Claims:

1. Isolated cytolytic T cell clone specific for a complex of an HLA-A2 molecule and one of: SEQ ID NO: 3, SEQ ID NO: 5, and SEQ ID NO: 9.

2. The isolated cytolytic T cell clone of claim 1, wherein said isolated cytolytic T cell clone is specific for a complex of an HLA-A2 molecule.

3. The isolated cytolytic T cell clone of claim 1, wherein said isolated cytolytic T cell clone is specific for a complex of an HLA-A2 molecule and SEQ ID NO: 5.

4. The isolated cytolytic T cell clone of claim 1, wherein said isolated cytolytic T cell clone is specific for a complex of an HLA-A2 molecule and SEQ ID NO: 9.

5. Method for inducing production of cytolytic T cells in a subject, comprising administering an amount of at least one of SEQ ID NO: 3, SEQ ID NO: 5, and SEQ ID NO: 9, to a subject who presents HLA-A2 molecule on cells, in an amount sufficient to provoke cytolytic T cell proliferation to complexes of HLA-A2 and one of SEQ ID NO: 3, SEQ ID NO: 5, and SEQ ID NO: 9.

6. The method of claim 5, wherein said subject is in need of cytolytic T cell proliferation.

7. Isolated peptide selected from the group consisting of: SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68, and SEQ ID NO: 69.

AMENDED CLAIMS

[received by the International Bureau on 7 January 1998 (07.01.98); original claims 1-7 replaced by new claim 1 (1 page)]

1. Isolated peptide selected from the group consisting of: SEQ ID NO: 67, SEQ ID NO: 68, and SEQ ID NO: 69.

FIG. 1

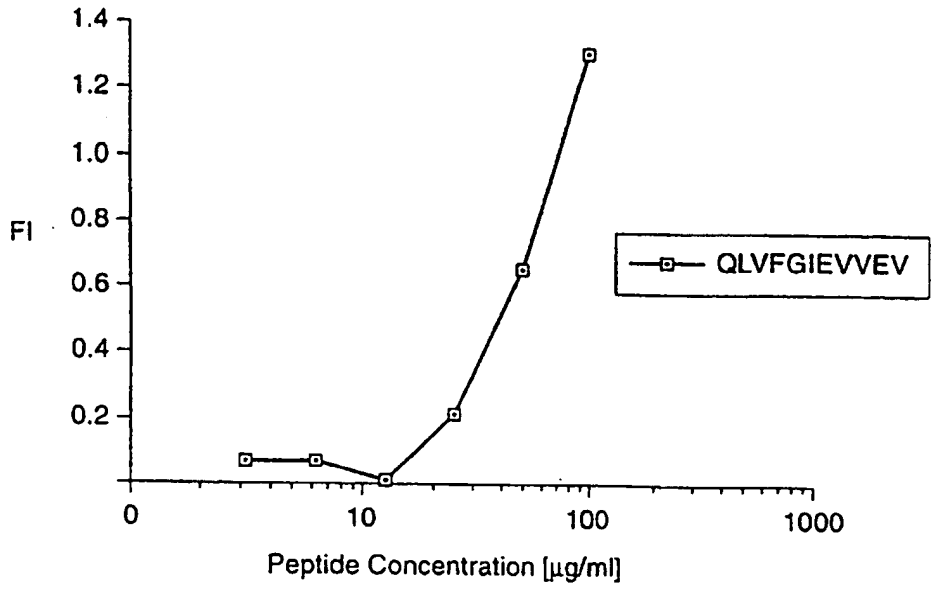

1.4
1.2
1.0
0.8
0.6
0.4
0.2
FI
0
10
100
1000
Peptide Concentration [µg/ml]
QLVFGIEVVEV

FIG. 2

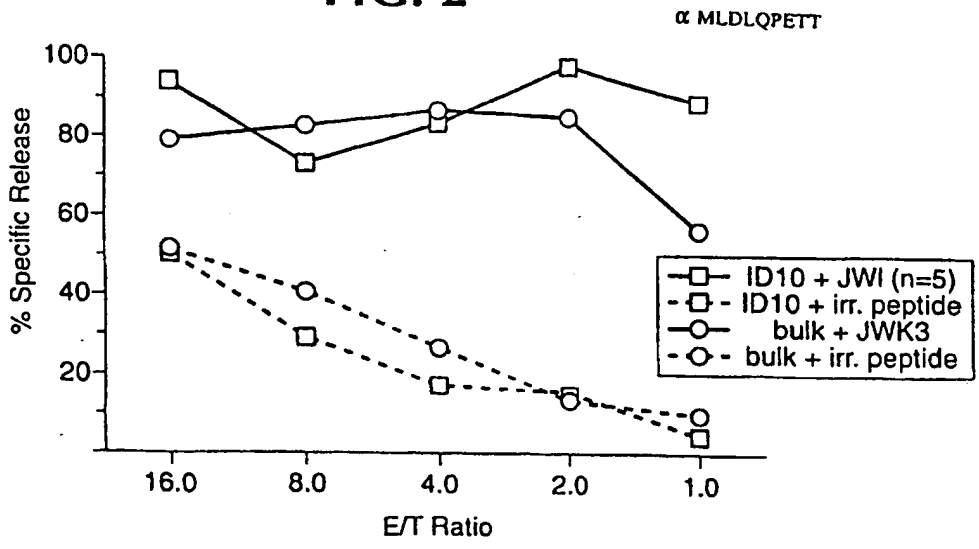

α MLDLQPETT
100
80
60
40
20
% Specific Release
16.0
8.0
4.0
2.0
1.0
E/T Ratio
ID10 + JWI (n=5)
ID10 + irr. peptide
bulk + JWK3
bulk + irr. peptide

FIG. 3A

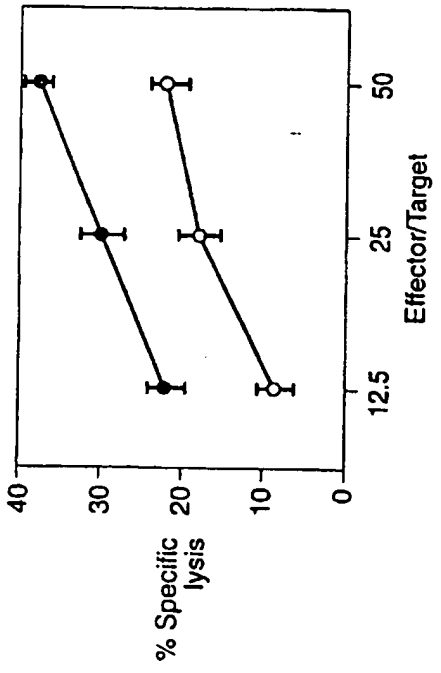


FIG. 3B

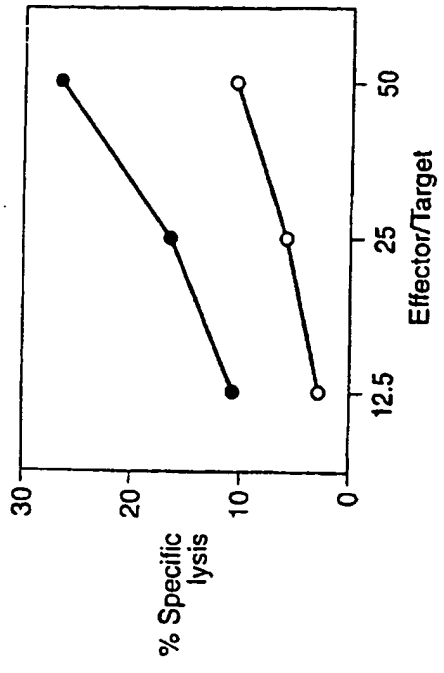


FIG. 3C

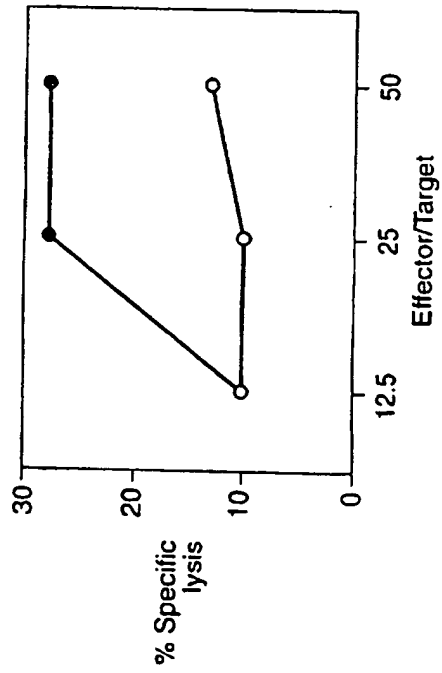
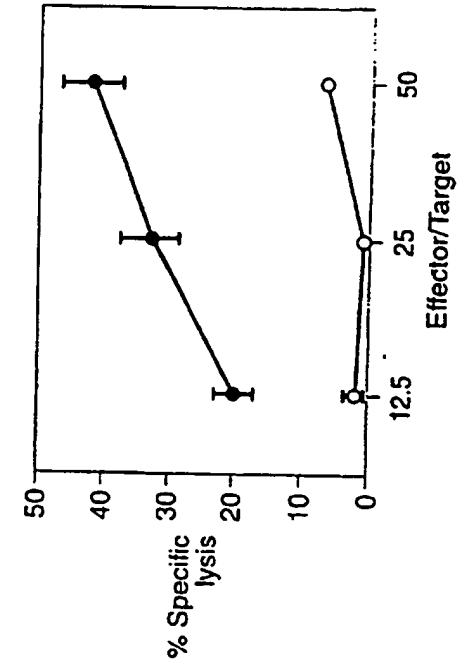


FIG. 3D



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/13002

A. CLASSIFICATION OF SUBJECT MATTER
IPC(6) :C07K 7/06; A61K 38/08
US CL :530/327, 328; 514/15; 424/185.1, 277.1
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 530/327, 328; 514/15; 424/185.1, 277.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
APS, STN-REGISTRY, CAPLUS
search terms: HLA-A2, MAGE 2; sequence search in registry file

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 95/25530 A1 (LUDWIG INSTITUTE FOR CANCER RESEARCH) 28 September 1995 (28.09.05), see entire document, especially claims 1-12.	1-7
X	WO 95/25740 A1 (LUDWIG INSTITUTE FOR CANCER RESEARCH) 28 September 1995 (28.09.95), page 9, line 24.	7
X	WO 94/20127 A1 (CYTEL CORPORATION) 15 September 1994 (15.09.94), page 86.	7
X	VAN DER BRUGGEN et al. A peptide encoded by human gene MAGE-3 and presented by HLA-A2 induces cytolytic T lymphocytes that recognize tumor cells expressing MAGE-3. Eur. J. Immunol. December 1994, Vol. 24, No. 12, pages 3038-3043, especially page 3041.	7

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

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"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search	Date of mailing of the international search report
03 NOVEMBER 1997	02.12.97

Name and mailing address of the ISA/US	Authorized officer
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Facsimile No. (703) 305-3230	Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/13002

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X, P	US 5,554,724 A (MELIEF ET AL) 10 September 1996 (10.09.96), see entire document.	1-7
X, P	US 5,591,430 A (TOWNSEND ET AL) 07 January 1997 (07.01.97), see entire document, especially column 5, line 5.	7
A	WO 95/22317 A1 (CYTEL CORPORATION) 24 August 1995 (24.08.95), see entire document.	1-7
A	WO 93/03764 A1 (CYTEL CORPORATION) 04 March 1993 (04.03.93), see entire document.	1-7

CORRECTED VERSION*

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08/687,226 25 July 1996 (25.07.96) US

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(74) Agent: LYNCH, John, E.; Felfe & Lynch, 805 Third Avenue, New York, NY 10022-7513 (US).

(81) Designated States: AU, CA, CN, JP, KR, NZ, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.
With amended claims.

(54) Title: ISOLATED PEPTIDES DERIVED FROM MAGE-2

(57) Abstract

New peptides derived from the MAGE-2 molecule and which bind to HLA-A*0201 molecules are disclosed. Some of these are especially useful because, when complexed to their HLA-A*0201 partner molecules, they induce CTL proliferation.

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BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon			PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		